Proposal Subject	NSSP Laboratory Evaluation Checklist
Specific NSSP Guide Reference	NSSP Guide Model Ordinance Chapter III. Laboratory @ .01 Quality Assurance D. (1)
Text of Proposal/ Requested Action	Chapter III@.01.D. (1) add the following to the end of the existing sentence (Laboratory evaluation criteria listed in Section IV Guidance Documents). The suggested NSP checklist is provided in the attached file.
Public Health Significance	An NSSP standardized NSP laboratory evaluation checklist will allow objective evaluation of laboratory conformance with NSSP requirements.
Cost Information (if available)	N/A
Action by 2003 Task Force I	Recommended that Proposal 03-107 be referred the appropriate committee as determined by the Conference Chairman.
Action by 2003 General Assembly	Adopted recommendation of 2003 Task Force I.
Action by USFDA	Concurred with Conference action.
Action by 2005 Lab Methods Review and Lab QA Committees	Recommended adoption of the revised Neurotoxic Shellfish Toxins (mouse bioassay) laboratory evaluation checklist as amended. The revised checklist is attached below.
Action by 2005 Task Force I	Recommended adoption of the Lab Quality Assurance Committee recommendation on Proposal 03-107.
Action by 2005 General Assembly	Adopted recommendation of 2005 Task Force I.
Action by USFDA	FDA concurs with adoption and incorporation of a Neurotoxic Shellfish Poisoning (NSP) laboratory checklist into the NSSP with the following changes. Changes 1 through 5 are editorial. Changes 6 and 7 are needed to restore universal applicability of the checklist to all laboratories involved in NSP testing.

1. Under the heading **Quality Assurance (QA) Plan** p. 33 add an item "*g. External performance assessment*". This was inadvertently left out of the Committee report and is consistent with both the Microbiology and PSP Checklists.
2. Under the heading **Digestion of Sample**, page 34, Item #2 add the word "*of*" before homogenized as an editorial change.
3. Under the heading **Digestion of Sample**, page 34, Item #3, add the word "*concentrated*" before HCl. This change is needed to be consistent with the official procedure which requires the use of concentrated (12 N) HCl.
4. Under the heading **Digestion of Sample**, page 34, Item #4, add the word "*for*" after the words gently boil as an editorial change.
5. Under the heading **Bioassay**, page 35, item #8.b, add the word "*test*" after the phrase "2 of 2" as an editorial change to distinguish the type of mice being used for determining the appropriate dilution for the toxin.
6. Under the heading **Work Area**, page 33, delete item #5. This item has no relevance outside of the FWRI toxin laboratory and as such needs to be deleted to maintain the applicability of the Checklist to all laboratories involved in NSP testing.
7. Under the heading **Extraction**, page 34, item #1, delete the remainder of the sentence after the word ventilation. This change does not alter the intent of item #1but, restores its applicability to all laboratories performing NSP bioassays without the benefit of an NSP dedicated laboratory as part of the facility.

DRAFT
Analysis for Neurotoxic Shellfish Toxins – Mouse Bioassay

~~* Indicates that this is not in the *Recommended Procedures*, 4th Edition~~

Weighted code		Item Description
		Quality Assurance (QA) Plan
C		1. Written Plan adequately covers the following (check those that apply): a. _____ Organization of the laboratory. b. _____ Staff training requirements. c. _____ Standard operating procedures. d. _____ Internal quality control measures for equipment, calibration, maintenance, repair and performance. e. _____ Laboratory safety. f. _____ ~~External FDA proficiency testing.~~ **Internal performance assessment.**
C~~*~~		2. QA Plan is implemented.
		Work Area
O		1. Adequate for workload and storage.
O~~*~~		2. Clean and well lighted.
O~~*~~		3. All work surfaces are nonporous and easily cleaned.
~~K*~~**C**		4. A separate, quiet area with adequate temperature control is maintained for acclimation and injection of mice.
C~~*~~		5. Following CIS guidelines, a closed system, e.g., room with adequate ventilation With explosion- proof electrical equipment and lighting has to be used for diethyl ether extractions. All electrical outlets and switches have to be on the outside of the room to avoid sparks and the fume hood should be without electrical service in the hood.
		Laboratory Equipment
K		1. The differing sensitivities in weight measurements required by various steps in the extraction procedure as well as the bioassay are met by the balances being used. a. _____ To determine sample weight, a sensitivity of at least 0.1 g at load of 100 g is required. b. _____ To determine the weight of the lipid extract and its subsequent volume adjustment, a sensitivity of at least 10 mg at loads of 1 and 10 g is required. c. _____ To determine the weight of the mice used in the bioassay, a sensitivity of 0.1 g at a load of 20 g is required.
O~~*~~		2. The calibrations of the balances are checked monthly using NIST Class S or ASTM Class 1 or 2 weights or equivalent. Records are maintained.
K~~*~~		3. The temperature maintained by the refrigerator is between 0 and 5°C.
O~~*~~		4. Refrigerator temperature is monitored at least once daily. Temperatures are recorded and records are maintained.
		Reagents
K		1. Concentrated (12N) HCl is used to acidify the homogenate.
O		2. Reagent grade NaCl is used in the extraction procedure.
C		3. Diethyl ether purified for lipid extraction is used for extracting lipids from the shellfish homogenates.
C~~*~~		4. Cottonseed oil (0.917 g/ml) or a solvent with a similar density **(0.915 to 0.927 g/ml)** is used as the toxin delivery system. Name of the solvent if substituted for cottonseed oil. **Specify** ~~D~~density _____
		Collection and Transportation of Samples
O~~*~~		1. Shellstock are collected in clean, waterproof, puncture resistant containers.
K~~*~~		2. Samples are appropriately labeled with the collector's name, the harvest area and the time and date of collection.
K~~*~~		3. Immediately after collection, shellstock samples are placed in dry storage between 0 and 10°C until analyzed.

K~~*~~		4. Shellstock samples are analyzed within 24 hours of collection or refrigerated unshucked until analyzed.
K~~*~~		5. Refrigerated storage of shellstock does not exceed 48 hours.
K~~*~~		6. If shellstock is refrigerated, only live animals are used in the analysis.
K~~*~~		7. If shellfish are shucked in a location other than the laboratory, they must be prepared according to steps 1- 9 in "Preparation of Sample" section below. ~~Samples are then double bagged.~~
		Preparation of Sample
C~~*~~		1. At least 12 animals are used per sample ~~and a minimum of 100 grams of meat~~.
O		2. The outside of the shell is thoroughly cleaned with fresh water.
K~~*~~		3. Shellstock are opened by cutting the adductor muscles.
C		4. Shell liquor is discarded.
O~~*~~		5. The inside of the shells is rinsed with fresh water to remove sand or other foreign material.
K~~*~~		6. Shellfish meats are removed from the shell by separating the adductor muscles and tissue connecting at the hinge.
K~~*~~		7. Damage to the body of the mollusk is minimized in the process of opening.
K		**8. 100 – 150 grams of meat are collected or all the available sample if there is less than 100 grams.**
O		~~8~~**9**. Shucked shellfish are drained on a #10 mesh sieve or equivalent without layering for 5 minutes.
K~~*~~		~~9~~**10**. Pieces of shell and drainings are discarded.
C		~~10~~**11**. Drained meats are blended at high speed until homogenous (60-120 seconds).
~~K*~~**C**		~~11~~**12**. Shellfish homogenates are digested ~~the same day they were blended~~**within 2 hours of blending**.
		Digestion of Sample
K~~*~~		1. All glassware used is clean and properly washed with a succession of at least three fresh water rinses, ~~1.2 N HCl~~, and a final distilled/deionized rinse to remove residual detergent.
K		2. 100 grams (or entire sample amount if less than 100 grams is available) homogenized sample is weigh~~t~~ed into a beaker.
C~~*~~		3. 1 ml of HCl and 5 g NaCl is added to the 100 gram homogenate and thoroughly mixed. (For samples <100 g, add reagents to obtain final concentrations of 0.12N HCl and 5% NaCl.)
C~~*~~		4. The homogenate is brought to a ~~rolling~~ boil and once 100 ± 1°C (sea level) is reached, gently boil ~~for a minimum of~~ 5 minutes ~~and until frothing ceases~~.
O~~*~~		5. The beaker is covered with a watch glass or equivalent during boiling to prevent excessive evaporation.
O~~*~~		6. The homogenate is boiled under adequate ventilation (fume hood).
O~~*~~		7. The boiled, acidified homogenate is cooled to room temperature or below in a refrigerator or in an ice bath.
		Extraction
C~~*~~		1. All steps in the extraction procedure which involve any manipulation of diethyl ether are carried out under adequate ventilation in a closed system that has explosion-proof electrical equipment and lighting following CIS guidelines. NO sparks.
C		2. 100 ml of diethyl ether is added to the cooled, acidified homogenate in a stoppered centrifuge tube and shaken vigorously for 5 minutes.
O		3. Centrifuge tubes are vented frequently while being shaken and before being centrifuged to avoid accidents.
C		4. The content of the centrifuge tubes are centrifuged at 2000 rpm for 10 to 15minutes.
C~~*~~		5. The clear upper ether phase is transferred to a large separatory funnel ~~or pre-weighed beaker. Any emulsion in the centrifuge bottle is excluded~~.
C~~*~~		6. The contents of the centrifuge tube are extracted three additional times for a total of four times, each time with 100 ml of diethyl ether. The upper phases

		are combined together in ~~either~~ the separatory funnel ~~or the pre-weighed beaker~~ (as in step 5).
C		7. ~~If a separatory funnel is used, t~~**T**he ether extract is transferred to a large, clean, dry pre-weighed beaker (~~first~~ discard~~ing~~ any emulsion or tissue that may have settled in the funnel.)
C		8. Ether is evaporated to dryness.
C		9. The final lipid residue is weigh~~t~~ed and the weight is recorded.
		Bioassay
C		1. The volume of the lipid residue is adjusted by weight to 10 ml (9.17 g) per 100 g shellfish extracted using cottonseed oil. If a solvent with a density similar to cottonseed oil is used, the volume is adjusted to a weight 10 times the density of the solvent. Specify the weight to which the volume is adjusted to. _____
K~~*~~		2. A 25 gauge hypodermic needle is used for injection.
C		3. Healthy male mice in the weight range of 17 to 23 grams from a stock colony are used for routine assays. Stock strain used _____. Source of the mice _____.
C~~*~~		4. Mice are allowed to acclimate for at least 24 hours prior to injection. In some cases up to 48 hours may be required. Typical length of the period of acclimation is _____.
O~~*~~		5. Mice are weighed to the nearest 0.1 gram.
C		6. The extract is completely mixed before it is injected.
C		7. Mice are injected intraperitoneally with 1 ml of the lipid extract.
C~~*~~		8. A total of 5 mice are injected with undiluted **or diluted** extract **as appropriate per sample in routine assays**. **a. The extract is not diluted when all test/assay mice survive beyond 110 minutes of injection.** **b. The extract is diluted when 2 of 2 mice or 3 of 5 assay mice survive for fewer than 110 minutes after injection.** **c. When dilution is required, only dilutions which produce mean/median death times within 110 to 360 minutes of injection are used in the analysis.**
C		9. The time of completed injection is recorded.
C~~*~~		10. Mice are continuously observed for at least 6 hours (360 minutes).
C		11. If death occurs within the period of continuous observation, the time of death to the nearest minute is noted by the last gasping breath.
K		12. If mice survive the test, the time of death is recorded as ">" the period of continuous observation.
		Calculation of Toxicity
C		1. The death time of each mouse is converted to mouse units (MU) using Table 8 in *Recommended Procedures*, 4th Edition.
O		2. Table 8 is interpolated for death times between 110 and 360 minutes that are not listed in the Table.
K		3. A weight correction in MU is made for each mouse injected using Table 8 in *Recommended Procedures*, 4th Edition.
O		4. Table 8 is interpolated to accommodate weights which are not listed.
C		5. The death time for each mouse in MU is multiplied by a weight correction in MU to give the corrected mouse unit (CMU) for each mouse.
C		6. The mean corrected mouse unit of the array of corrected mouse units (CMU) is used when all the mice injected with diluted or undiluted extract die during the period of continuous observation.
C		7. The median corrected mouse unit of the array of corrected mouse units (CMU) is used when at least one mouse either survives the test or dies.
C		8. The concentration of toxin is determined by the formula: Mean or median CMU x Dilution Factor x 10.
C		9. When the time of death is known for certain for all mice injected, toxicity is determinate and the toxin concentration is reported as the number of mouse units per 100 grams of sample.